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Effectiveness of catechin extract of green tea (camellia sinensis) on porphyromonas gingivalis

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Abstract

Objective: To find out the effectiveness of catechin extract of green tea (Camellia sinensis) on Porphyromonas gingivalis.

Material and Methods: The independent variables were catechin extracts of green tea (Camellia sinensis) starting from the 40%, 20%, 10%, 5%, 2.5% and 1.2% concentrations. The dependent variables were the Minumum Inhibitory Concentration (MIC) and the Inhibition zone of bacterial growth of Porphyromonas gingivalis. Control variables were time, culture medium, and temperature. The sample of research were Porphyromonas gingivalis which has been bred and catechin extract of green tea (Camellia sinensis) that extracted by maceration method..

Results: At the 40%, 20%, 10%, and 5% concentrations, the growth of Porphyromonas gingivalis did not change. As for the 2.5% and 1.25% concentrations, bacterial growth has occurred.

Conclusion: Catechin extract of green tea (Camellia sinensis) was effective in inhibition of Porphyromonas gingivalis.

Keywords: Camellia sinensis, Catechin extract, Porphyromonas gingivalis

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Introduction

Green tea has received more special attention in the last two decades due to its favorable results for human health problems. Green tea was one of the herbal plants. Herbal plants had different types of active component compositions when planted in different places, geographical locations, forms of presentation and times. These affected the determination of antibacterial activity.[1]

This antibacterial activity is affected by the concentration of polyphenols in green tea extracts. The Minimum Inhibitory Concentration of polyphenols was 0.25-1mg/ml. Polyphenols of green tea were effective in inhibiting the growth of bacteria that caused periodontium diseases, namely Porphyromonas gingivalis and cariogenic bacteria such as streptococcus mutans and streptococcus sobrinus. The use of tea polyphenol mouth rinses with 0.05% or more concentration has also been proven to inhibit the formation of dental plaque.[2]

The 0.5% concentration of green tea extract had a greater effect on increasing salivary volume. Green tea was also alkaline and bitter-tasted. The alkaline nature of green tea maintained the acid-base balance of body fluids. The bitter taste of green tea due to the presence of catechins. It stimulated salivary secretion. The total polyphenols in green tea were 10.81% of the dry weight of tea leaves, while the total polyphenols in solid extracts of green tea ranged from 37-56% of the dry weight.[2]

Green tea contained polyphenols with catechin compounds, the highest content of catechin in green tea was EGCG, around 50-80%. EGCG actively suppressed the occurrence of inflammation, killed and inhibited various microorganisms, and had the ability as an antioxidant. The polyphenols content of 100 grams of green tea leaves obtained about 25%. The EGCG contained in green tea had an antibiotic effect that worked directly by way of antibiotics that damaged cell membranes in bacteria, inhibited the existing synthesis of fatty acids, and inhibited the activity of enzymes present in bacteria.[3]

Polyphenols were antibacterial substances that are proven to be able to maintain the body's immune system by phagocytosis of bacteria or other foreign substances that entered the body. This polyphenol content is used to kill the bacteria porphyromonas gingivalis which caused periodontitis.[4]

Based on the background described above, we aimed to find out the effectiveness of catechin extract of green tea (camellia sinensis) on porphyromonas gingivalis.

Material and Methods

The type of research used in this study was an experimental laboratory research. The research used

was a posttest only control group design using the dilution method and disk-diffusion method. The study was conducteded in June 2020 at the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University, and PT IFI South Sulawesi.

There were three variables in this study. The independent variables were catechin extracts of green tea (camellia sinensis) starting from the 40%, 20%, 10%, 5%, 2.5% and 1.2% concentrations. The dependent variables were the Minumum Inhibitory Concentration (MIC) and the Inhibition zone of bacterial growth of porphyromonas gingivalis. Control variables were time, culture medium, and temperature. The sample of research were Porphyromonas gingivalis which has been bred and catechin extract of green tea (camellia sinensis) that extracted by maceration method.

The tools were analytical scales, test tubes, test tube racks, glass jars, petri dishes, micropipettes, paper disks, caliper, measuring cups, incubator, autoclaves, tweezers, bunsen, filter paper, Erlenmeyer flasks, 1 mL spoit, paper label, funnel, handscoon and mask, scrub (mac carymey) bottle, water bath and vortex. The materials were Porphyromonas gingivalis isolates, catechin extract of green tea (Camellia sinensis), amoxicillin, Muller Hinton Agar (MHA) medium, DMSO (Dimethyl sulfoxide), spritus, Mc farland 0.5 (diluted), aluminum foil, and sterile aquades.

Petri dishes are wrapped in aluminum foil. Erlenmeyer flask is filled with 250 ml of aquades and then covered with compacted cotton. All tools are sterilized in an autoclave at 121°C for 2 hours.

Fresh green tea (camellia sinensis) leaves are washed and dried in a drying cupboard at 45°C for 48 hours. After drying, pollinated using a pollinating machine with a hole diameter of 1 mm until finished and weighed with a balance sheet so that the dry weight is obtained. Then the extraction process is carried out using a water solvent with a high-pressured extraction technique. The pollen is put into a jar containing 70% ethanol while stirring for 30 minutes and allowed to stand for at least 1-2 hours.

Table 1 **Results of the measurement of the inhibition zone diameter (mm) on porphyromonas gingivalis**

Type of intervention	Concentration (%)	I	II	III	Mean
Catechin extract of green tea	5	6.9	8	8.1	7.67
		9.2	9	10.3	9.50
	20	13.3	12.4	12.6	12.77
	40	15.5	16	17	16.17
Control (+) Amoxicillin		25.4	26.7	27.9	26.67
Control (-) DMSO		6.3	6.3	6.4	6.33

Results

The result is filtered 3 times with a Buchner funnel to obtain filtrate and residue. The filtrate obtained was evaporated using a vacuum rotary at 70°C and heated with a water bath so that a thick extract was obtained. This thick extract is poured into a porcelain cup and heated again with a water bath while continuing to stir. The end result is a green tea extract with a concentration of 100%. Pure green tea extract was diluted using in sterile aquades in accordance with predetermined concentrations, namely 40%, 20%, 10%, 5%, 2.5% and 1.5%.

The dilution of green tea extract was the 40%, 20%, 10%, 5%, 2.5% and 1.5% concentrations. Each bottle was given 1 ml of green tea stock solution pipetted into a sterile petri dish. Then six bottles are added 9 ml of sterile MHA (Muller Hilton Agar) which is still melting. After it cools, each petri dish is scraped with bacterial suspensions equivalent to 105 (108 Mc Farland 0.5 bacterial stock) diluted to 105. All Petri dishes were incubated at 37oC for 24 hours in anaerobic conditions. The Minimal Inhibitory Concentration is calculated as the smallest concentration that did not show bacterial growth. After table 1 Results of the measurement of the inhibition zone diameter (mm) on Porphyromonas Gingivalis.

From table 1, descriptively is seen that the 10% concentration of catechin extract of green tea has the smallest mean of the inhibition zone (9.5 mm) when compared to the entire concentrations of catechin extract of green tea. The 40% concentration of catechin extract of green tea has the biggest mean of the inhibition zone (16.17 mm). lin buffer solution and taken to the Anatomic Pathology Laboratory of the Faculty of Medicine, Universitas Gadjah Mada to be processed up to the immunohistochemical staining (MMP-9polyclonal antibody) and PAS staining. With the Optilab camera, microscopic images were taken on the results of the preparation. MMP-9expression data and epithelial thickness were calculated with Image-J software combined with direct observation by anatomical pathologists figure 1A and figure 1B.

After the data normality test with the Shapiro-Wilk test is performed, the DMSO (negative control) group showed a p-value <0.05 so that the data were not normally distributed. After the data transformation is performed, a p-value <0.05 is still obtained in the DMSO (negative control) group, which means the group is not normally distributed. Therefore, further hypothesis test cannot be performed with the One-Way ANOVA and Post Hoc LSD (Least Significant Difference) parametric tests, but instead using

Table 2 Results of statiscal test of the inhibition zone on porphyromonas gingivalis

Type of intervention	Concentration (%)	N	Normality Test*
Catechin extract of green tea	40	3	0.637
	20	3	0.407
	10	3	0.274
	5	3	0.144
Control (+) Amoxicillin		3	0.956
Control (-) DMSO		3	0.000

*Shapiro Wilk test: p > 0.05; normally distributed data.

Table 3 Results of Kruskal-Wallis Test

The null hypothesis	Test	p value	Conclusion
Catechin extract of green tea cannot inhibit the growth of porphyromonas gingivalis	Kruskal Wallis*	0.005	The null hypothesis was rejected

* Kruskal-Wallis test: p > 0.05; the null hypothesis is accepted

Table 4 Results of Post Hoc Mann-Whitney statistical test of the inhibition zone on Porphyromonas gingivalis (the 40% concentration)

Treatment group	Comparison	p-value
Concentration 40%	20%	0.05
	10%	0.05
	5%	0.05
	K(+)	0.05
	K(-)	0.46

Table 5 Results of Post Hoc Mann-Whitney statistical test of the inhibition zone on Porphyromonas gingivalis (the 20% concentration)

Treatment group	Comparison	p-value
Concentration 20%	40%	0.05
	10%	0.05
	5%	0.05
	K(+)	0.05
	K(-)	0.46

alternative tests, namely the Kruskal-Wallis and Post Hoc Mann-Whitney non-parametric test table 2.

Table 3 in the Kruskal-Wallis statistical test, p=0.005 (p<0.05) is obtained, which means the alternative hypothesis is accepted. So, it can be concluded that the catechin extract can inhibit the growth of porphyromonas gingivalis as indicated by the presence of the inhibition zone diameter in different treatment groups according to the concentration of the catechin extract. To find out which group concentrations had differences, a Post Hoc analysis must be performed. The Post Hoc analysis used for the Kruskal-Wallis test was the Mann-Whitney test.

In the Mann-Whitney test, $p \leq 0.05$ is obtained for comparison between concentrations and comparison between concentrations of catechin extract of green tea with Amoxicillin (positive control) and DMSO (negative control), so alternative hypotheses are accepted which means there were significant differences between groups. The results of Post Hoc Mann-Whitney Test can be seen in the following table 4.

Table 4 at the 40% concentration of catechin extract of green tea when compared with the 20%, 10%, 50% concentration, positive and negative controls had $p \leq 0.05$ which means there was a significant difference or had a different effect.

Table 5 at the 20% concentration of catechin extract of green tea when compared with the 40%, 10%, 5% concentration, positive and negative controls had $p \leq 0.05$ which means there was a significant difference or had a different effect.

Discussion

In the inhibition zone test using catechin extract of green tea (camellia sinensis) on the growth of porphyromonas gingivalis, it was seen that the inhibition zone formed is increased in proportion to the increasing concentration of the extract. Camellia Sinensis had many useful phytochemical compounds. This was due to green tea (camellia sinensis) leaves contained tannins, flavonoids, polyphenols, and methylxantyl compounds.[5]

Phenol compounds are generally known as disinfectants used to kill pathogenic microorganisms. Flavonoids were the largest phenol compounds in nature and have been known to have biological activities as antioxidants, antimelanogenesis and antimutagenesis. Polyphenol compounds have been shown to have antibacterial activity. In addition, the leaves also contained essential oils and also coumarin.[6-8] Camellia sinensis, is known to have a good antibacterial effect. Suggested that each 100 gr of tea leaves contained 17 kJ calories, 75-80% water, 25% polyphenols, 20% protein, 4% carbohydrates, 2.5 - 4.5% caffeine, 27% fiber, and 6% pectin. The other contains were solids and consisted of organic and inorganic. The most important organics in its process included polyphenols, carbohydrates, and their product, nitrogen bonds, pigments, enzymes,

and vitamins. Polyphenols or catechins in green tea were antibacterial substances that have been proven to be able to maintain the body's defenses by phagocytosis of bacteria or foreign substances that enter the body.[9-12] The green tea has been widely studied in the field of dentistry as an antibacterial.

This study proved that extract of green tea (camellia sinensis) had a bacterial inhibition on porphyromonas gingivalis with the 10% concentration had the smallest mean of inhibition zone (9.5mm) when compared to the entire concentrations of extract of green tea (camellia sinensis). The zone of inhibition at the 40% concentration had the biggest inhibition zone (16.17 mm).[13-15]

At the 40% concentration of catechin extract of green tea when compared with the 20%, 10%, 5% concentrations, positive and negative controls had $p \leq 0.05$ which means there was a significant difference or had a different effect.[16]
Thus, the bacterial activity only can be observed in diffusion methods to see inhibition zones of the extract of green tea (camellia sinensis). The 40% concentration had the biggest inhibition zone (16.17mm) and the inhibition zone was greater than the positive control (amoxicillin) which is used as a comparative antibiotic of green tea (camellia sinensis) extract. This study has proven that green tea (camellia sinensis) extract was effective in the inhibition of Porphyromonas gingivalis. Thus, the hypothesis was accepted.[17]

Conclusion

Catechin extract of green tea (camellia sinensis) was effective in inhibition of porphyromonas gingivalis. The 5% concentration of green tea (camellia sinensis) extract had smallest mean of the inhibition zone (7.67 mm) when compared to the entire concentrations of green tea (camellia sinensis) extract. The 40% concentration of green tea (camellia sinensis) extract had greatest inhibition zone (16.17 mm). Minimal Inhibitory Concentration (MIC) of each green tea extract from 1.5% - 2.5% concentrations continued to occur bacterial growth and the 5%, 10%, 20%, and 40% concentrations did not occur bacterial growth.

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Conflict of Interest

The authors report no conflict of interest.

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